QUANTITATION USING PLEXOR HY

A. SCOPE

The Plexor HY System is a real-time PCR assay to determine the concentration of total human DNA and male human DNA simultaneously in one reaction. The kit contains an internal PCR control (IPC) to test for false-negative or inaccurate results that may occur in the presence of PCR inhibitors and a melt curve function to confirm that the correct product was amplified.

The Plexor HY System works by measuring a reduction in fluorescent signal during amplification. Amplification of each target uses only two primers, one of which contains both a fluorescent tag and a modified base. As amplification proceeds, fluorescence is reduced by site-specific incorporation of a fluorescent quencher opposite the complementary modified base. The quencher is in close proximity to a fluorescent dye located on the end of the primer, resulting in a reduction of fluorescent signal. After PCR, a melt analysis can be performed to provide an internal control for the final assay design or to expedite troubleshooting.

B. QUALITY CONTROL

B.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure to prevent contamination.

B.2 Decontaminate the bench work area with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner, before and after quantitation set up.

B.3 See DOC ID 1835 to determine reagent expiration dates.

B.4 Each new lot of Plexor HY must undergo quality control testing prior to being used for the quantitation of casework samples.

Quality Control Testing: A standard curve using the reagents from the new lot of Plexor HY will be prepared along with a standard curve prepared using the previously quality control tested kit. At least two non-probative samples (including at least one male sample) and a no-template control (NTC) will be quantitated using the new reagents and the old reagents. The samples quantitated using the new reagents will be analyzed using the new standard curve and the samples quantitated using the old reagents will be analyzed using the old standard curve. Samples used as part of the quality control testing should have a concentration of less than 50 ng/µL. Samples may be diluted to achieve a concentration of less than 50 ng/µL. Results from the same non-probative sample should be within 50% of each other. For example, if a sample is 20 ng/µL when quantitated using the old reagents and standard curve then the same sample quantitated using the new reagents and standard curve should be no less than 10 ng/µL and no greater than 30 ng/µL. The "Quant

Kit QC Calculator" (DOC ID 15365) should be used for this calculation. The NTC must be free from contaminants.

B.5 Each new lot of TE^{-4} must undergo quality control testing prior to being used to dilute casework samples.

Quality Control Testing: A known sample will be diluted with the TE^{-4} undergoing quality control testing along with an amplification negative control containing 17.5µL of TE^{-4}. These samples will be carried through autosomal amplification and electrophoresis. The TE^{-4} will pass quality control testing when a good quality DNA profile with the correct results is obtained for the diluted sample, as described in the GlobalFiler interpretation guidelines (DOC ID 12628) and the amplification negative control is free from contaminants. The quality control data will be placed into the critical reagent binder.

B.6 At least one negative quantitation control must be quantitated on each quantitation plate.

B.7 Optical plates should be kept in the appropriate base at all times during plate setup and centrifugation. This limits the amount of debris introduced into the AB 7500 instrument and prevents damage to the plate wells that may interfere with the optical readings.

B.8 Quantitation setup must be performed in the pre-amplification room. Do not bring quantitation trays into the post amplification room.

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Treat all biological specimens as potentially infectious.

C.4 Distinguish all waste as general, biohazard, or sharps and discard appropriately. The heat block of the AB 7500 can become very hot. Be careful not to touch the heating surfaces during plate loading and unloading.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 Plexor HY Quantitation Kit (Promega)

D.2 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

D.3 70% Reagent Alcohol (Decontamination)

D.4 TE^{-4} (10mM Tris-HCl- 0.1mM EDTA, 1L)

Add 10mL 1 M Tris-HCl, pH 8 and 150µl 0.5 M EDTA to 990mL deionized water. Store at room temperature.

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 AB 7500 Real-Time PCR instrument and software
E.1.2 Microcentrifuge
E.1.3 Pipettes
E.1.4 Vortexer
E.1.5 96 well plate centrifuge

E.2 Supplies

E.2.1 Kimwipes
E.2.2 Sterile aerosol-resistant tips
E.2.3 Microcentrifuge tubes racks
E.2.4 AB optical 96-well plates
E.2.5 AB optical adhesive covers
E.2.6 Adhesive seal applicator
E.2.7 96-well plate base
E.2.8 Disposable gloves
E.2.9 Scrubs
E.2.10 Lab coat
E.2.11 Mask
E.2.12 Eye protection (e.g. safety glasses, face shield)
E.2.13 DNA Analysis Workbook (optional)

F. PROCEDURE

F.1 Thaw the Plexor HY Male Genomic DNA Standard (50 ng/µL) overnight at approximately 4°C. After initial thawing, store at approximately 4°C.

F.2 Vortex the DNA standard for at least 10 seconds. Prepare a serial dilution of the Plexor HY Genomic DNA Standard as indicated below, vortexing each dilution for at least 10 seconds before removing an aliquot for the next dilution. These standards may be used by multiple analysts for up to 24 hours after they are prepared.

Serial Dilution of the Plexor HY Genomic DNA Standard

Concentration	Volume of DNA	Volume of TE^{-4} Buffer
50 ng/µL	Use undiluted DNA	0 µL
10 ng/µL	5 µL of undiluted DNA	20 µL
2 ng/µL	5 µL of 10 ng/µL dilution DNA	20 µL
0.4 ng/µL	5 µL of 2 ng/µL dilution DNA	20 µL
0.08 ng/µL	5 µL of 0.4 ng/µL dilution DNA	20 µL
0.016 ng/µL	5 µL of 0.08 ng/µL dilution DNA	20 µL
0.0032 ng/µL	5 µL of 0.016 ng/µL dilution DNA	20 µL

F.3 Thaw the Plexor HY 2X Master Mix, Plexor HY 20X Primer / IPC Mix, and Amplification Grade Water at room temperature. The Amplification Grade Water may be stored in the refrigerator.

F.4 Briefly vortex the Plexor HY 2X Master Mix and Plexor HY 20X Primer / IPC Mix for at least 10 seconds to mix. **Do not** centrifuge after vortexing, as this may cause the primers to be concentrated at the bottom of the tube.

F.5 Determine the number of reactions to be set up. Include two no-template control (NTC) reactions for each set of reactions. Add additional reactions to this number (usually 3 to 5) to compensate for loss during pipetting. It is critical that the same reaction mix is used for the entire run.

F.6 Prepare the reaction mix by combining the Amplification Grade Water, Plexor HY 2X Master Mix, and Plexor HY 20X Primer / IPC Mix as indicated in the below template. The same reaction mix will be used during the quantitation of the NTCs, samples, and DNA standards.

Preparation of Reaction Mix for Quantitation Assays (assumes 2 µL of template DNA per reaction).

Component	Volume (Per Reaction)
Plexor HY 2X Master Mix	10 µL
Water, Amplification Grade	7 µL
Plexor HY 20X Primer / IPC Mix	1 µL
Final volume	18 µL

F.7 Vortex briefly to mix.

F.8 Add 18 µL of the reaction mix prepared in Step F.6 to each of the appropriate wells of an optical-grade PCR plate.

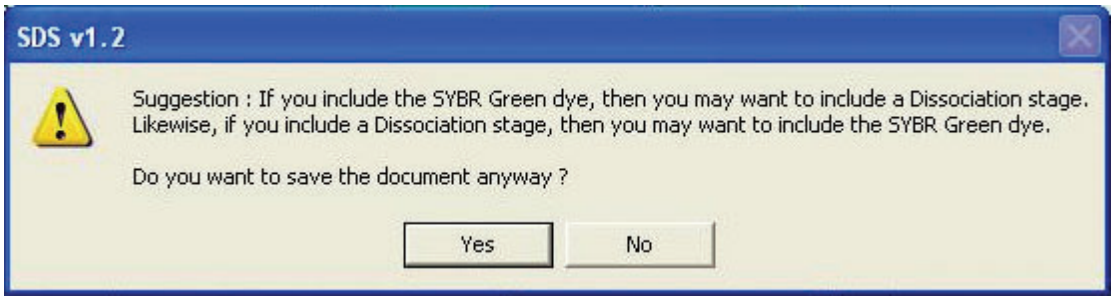
F.9 Add 2 µL of DNA standard or unknown sample to the reaction mix in the appropriate wells. Add 2 µL of TE^{-4} to the reaction mix in the NTC wells. Duplicate amplification of standards is required.

F.10 Seal the plates with an optical adhesive cover using the plate cover applicator.

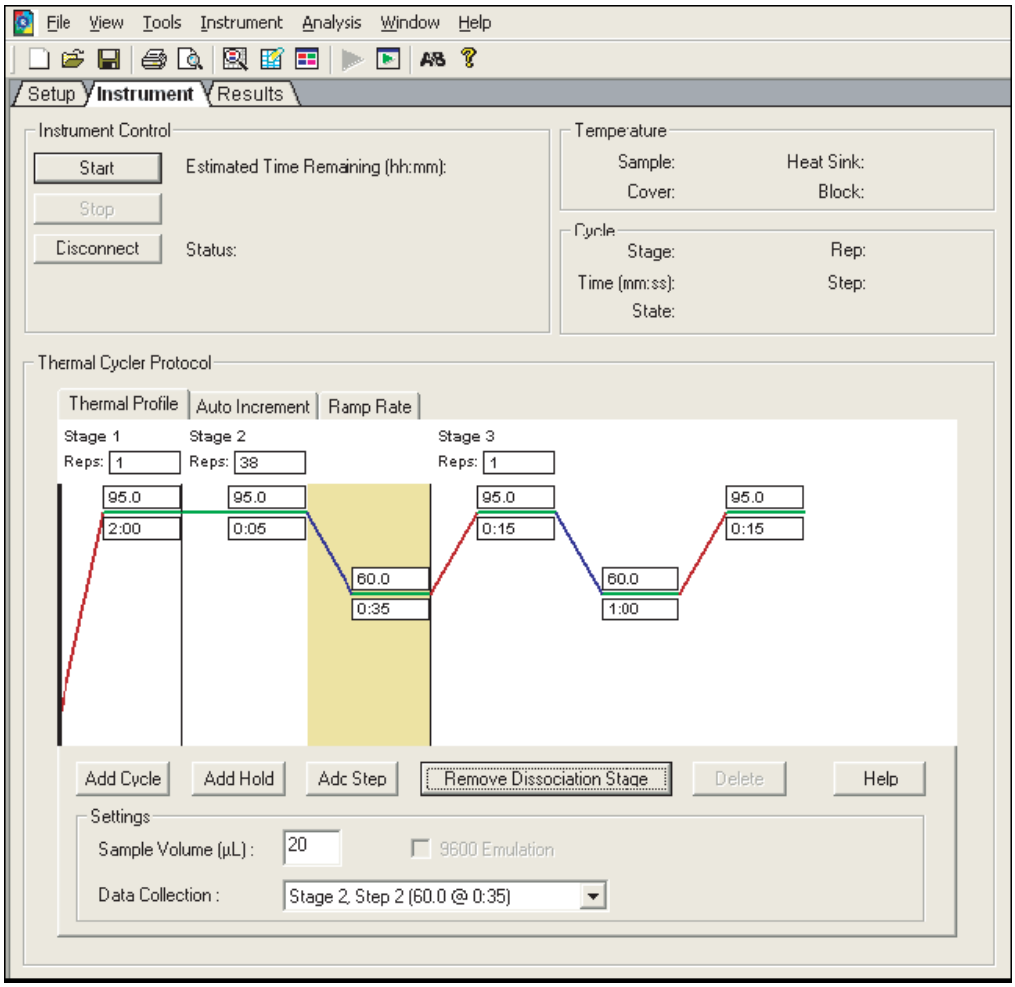
F.11 Centrifuge the plate and base briefly to collect the contents of the wells at the bottom. The plate is ready for thermal cycling. Protect the plate from extended light exposure or elevated temperatures before cycling. Handle the plate by the edges, and avoid touching the bottom of the plate.

F.12 Position the plate in the instrument thermal block so the Well A1 is in the upper-left corner. The notched corner of the plate is in the upper-right corner.

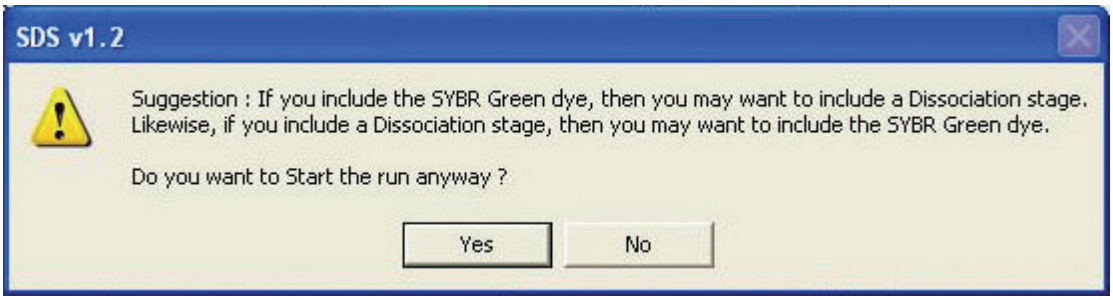
F.13 In the SDS software open the plate document that was set up for a Plexor HY run (See Plexor HY instruction manual for generation of new template if needed). Uncheck wells that are not being used. Alternatively, unused wells may be "omitted" prior to analysis. Save the file as an .sds document. The following message will appear when saving the document. Click "Yes".



F.14 Select the **Instrument** tab. The following cycling parameters are displayed:



F.15 Click **Start.** The following message will appear when starting the instrument. Click "Yes".



G. INTERPRETATION GUIDELINES

G.1. See Analysis of Plexor HY Data procedure.

H. REFERENCES

H.1 www.promega.com/plexorhy/

H.2 Plexor HY System for the Applied Biosystems AB 7500 and AB 7500 FAST Real-Time PCR Systems, Instructions for use of products DC1000 and DC1001.